(An Association Unifying the Sciences, Engineering, and Applied Research)
ISSN (Online): 2279-0055

International Journal of Emerging Technologies in Computational and Applied Sciences (IJETCAS)

www.iasir.net

Effect of antibiotics on varied density of septicemia causing bacteria of silkworm

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Abstract: *Antibiotics are widely used in sericulture industry for mitigating losses caused by bacterial septicemia among microbial disease. The use of standardized antibiotic concentration for different densities of bacteria by Kirby-Bauer disk diffusion method determines minimum inhibitory concentration for septicemia bacteria. Inferences obtained by impregnating standard antibiotics like Ampicillin (25 mg), Gentamycin (30 mg) and Kanamycin (30 mg) through disk diffusion method shows that maximum inhibition in Gentamycin (30 mg), intermediate inhibition zone in Kanamycin (30 mg) and lowest or no inhibition zone for β-lactamic Ampicillin (25 mg) are shown. Insights into standard antibiotic susceptibility concentration of septicemia agents augment silk production by maximizing utilization of food and lowers development of antibiotic resistant septicemia bacteria. Advance investigation into an area under disk diffusion yields Minimum Inhibitory Concentration (MIC) for human infection from these opportunistic pathogens.*

Keywords: *Kirby-bauer, Septicemia, Minimum Inhibitory Concentration.*

I. Introduction

Diseases in silkworm cause 10 to 20 percent loss in sericulture productivity. Bacterial diseases in silkworm are generally classified as Bacterial flacherie, bacterimia, septicemia and toxicosis (Ponnuvel & Yamakawa, 2002). Bacterial flacherie is caused by species of Streptococci, Staphylococci, Bacillus and Serratia(Subramanian, Mohanraj, & Muthuswamy, 2009). The pathogenic bacteria *Serratia* and *Bacillus Sp*, invade the haemocoel of the silkworm, multiply and cause Septicemia. Antibiotics are widely used in sericulture industry as a component as therapeutic applications and as bed disinfectants against bacterial diseases(Souad M. Mahmoud, Rehab H. Taha and Saad, 2012). Several studies indicated that gut micro flora is sensitive to the antibiotics, which cause adverse effects on the physiological system of the insects (Freitak, Heckel, & Vogel, 2009). Administration of antibiotic was reported to have detrimental effect on intestinal microflora of silkworms. Chloromycetin administration cause general reduction in gut bacterial population of silkworms. It was observed that population of endogenous gut bacteria were reduced in number (Subramanian et al., 2009). There is increasing concern about the resistance of microorganisms to various drugs and the perspective of continuous use of antibiotic finally leading to death of the organism is not yet well defined (Henrique Douglas Melo Coutinho, et al., 2008). Therefore, many measures to solve this problem need to be adopted, e.g., the controlled use of antibiotics, expansion of research for the better understanding of the resistance mechanisms, and continuing attempts to develop new synthetic and natural drugs.
The dynamics of the disk diffusion technique, in which the size of the zone is related to a critical concentration of antimicrobial agent, a critical population of organisms and a critical time, makes a suitable method for testing silkworm sensitive resistant micro-organisms and concentration of antibiotics to be administered.

II Materials and methods

The larvae were anesthetized with 70 percent chloroform, surface sterilized with 70 percent alcohol and alimentary canal was dissected out and transferred to sterilize distilled water. After isolation, the gut was homogenized immediately in 0.1 M Potassium phosphate buffer at pH 7.0(Subramanian et al., 2009).
Diseased dead and live silkworm larvae with known septicemia symptoms were collected. Live larvae anaesthetized and surface sterilized with 70 percent alcohol, haemocoel of larvae were collected by piercing larvae skin through sterilized needle and is serially diluted using distilled water, plated onto Nutrient agar and incubated for 72 - 96 hrs(Kumar & Ramakrishna, 2013).

Antibacterial sensitivity assay

The antibiotic sensitivity to Serratia and Bacillus sp was determined using the Kirby-Bauer disk diffusion method with minor modifications (Garner & Rosner, 1975) and used to test the susceptibility of the *Serratia* and Bacillus *sp.*, isolates of silkworm pathogen using different antimicrobial agents Ampicillin (25 mg), Gentamycin (30 mg) and Kanamycin (30 mg) (Button, et al., 1975). The inocula for testing microorganism were prepared by

growing *Serratia marcescens*, *Serratia plymuthica* and *Bacillus cereus* on separate agar plates and 18 hr colonies from the plate were transferred with inoculating loop into 3 ml of phosphate saline buffer of pH 7 into a test tube to form stock inoculums of known density by enumeration method.

Stock sample with known density taken and different aliquots of 0.1, 0.2, 0.3 and 0.4ml of samples were poured into 20 ml solidified Muller-Hinton agar plates, by using sterile glass rod inoculums was spread and incubated for 18 hr to obtain a uniform growth of bacteria. Antibiotic disks were placed onto the surface of lawn of bacteria with proper spacing using forceps and pressed a little to facilitate proper diffusion and incubated at 30±1ºC for 18 h. After 18 h incubation antibiotics to which organisms are sensitive, formed clear zone around it, and to which organisms are resistance they do not form any zone of inhibition around the disk. Those that are intermediate formed little zone of inhibition. The diameter of inhibition including the disk diameter of 9mm was measured by using graduated scale. The activity antibiotic disks were calculated for each septicemia pathogen of silkworm (A.Maiti, 2013).

III. RESULTS

The Kirby-Bauer disk diffusion method employed to determine antibiotic susceptibility for Serratia and Bacillus species. These bacterial cultures of 0.1, 0.2, 0.3 and 0.4 aliquots were spread on 20 ml of Muller Hinton pre-made agar plates and incubated for 24 hr at 37˚c. Uniformly grown confluent bacterial lawn plates selected and antibiotic disks of Ampicillin, Gentamycin and Kanamycin were impriginated in appropriate distances and incubated for 18 hr at 37˚c. The antibiotic disks of Gentamycin (30 mg), Kanamycin (30mg) formed inhibition zones on Gram negative (G-ve) and Gram positive (G+ve) bacteria, but Ampicillin (25 mg) showed inhibition zone on plates of *Bacillus cereus* (G+ve) bacteria and lower inhibition zones on *Serratia plymuthica* (G-ve) bacteria. Ampicillin (25 mg) shows no bactericidal activity for *Serratia marcescens*, slight activity for *Serratia plymuthica* and *Bacillus cereus.* Gentamycin(30 mg) shows activity on all tested bacteria with maximum inhibition on *Serratia* species. Kanamycin (30 mg) forms inhibiton zones on all tested bacteria. The results for experiment are summarized in Table 1and graph.

Table 1: Effect of antibiotics on growth of Silkworm septicemia causing bacteria.

Name of the organism	Stock solution taken in ml	Gentamycin(30mg) Inhibition zone in mm	Kannamycin(30mg) Inhibition zone in mm	Ampicillin (25mg) Inhibition zone in mm
Serratia marscenes	0.1	28.8 ± 1.5	26.8± 1.5	0
Serratia marscenes	0.2	27.6 ± 1.5	15.2± 1.5	0
Serratia marscenes	0.3	29.2± 1.5	13.2± 1.5	0
Serratia marscenes	0.4	26.8± 1.5	12.8± 1.5	0
Serratia plymuthica	0.1	27.6 ± 1.0	24.4± 1.0	8.9± 1.0
Serratia plymuthica	0.2	27.6± 1.0	13.2± 1.0	8.8± 1.0
Serratia plymuthica	0.3	24.4± 1.0	10.8± 1.0	8.4± 1.0
Serratia plymuthica	0.4	26± 1.0	18± 1.0	8± 1.0
Bacillus cereus	0.1	23± 1.0	13± 1.0	11± 1.0
Bacillus cereus	0.2	22.4± 1.0	12.4± 1.0	0
Bacillus cereus	0.3	20.4± 1.0	12± 1.0	9.6± 1.0
Bacillus cereus	0.4	20± 1.0	11.6± 1.0	10.4± 1.0

Mm =millimeter, ml= milliliter, ± =variation in between.

IV. DISCUSSION

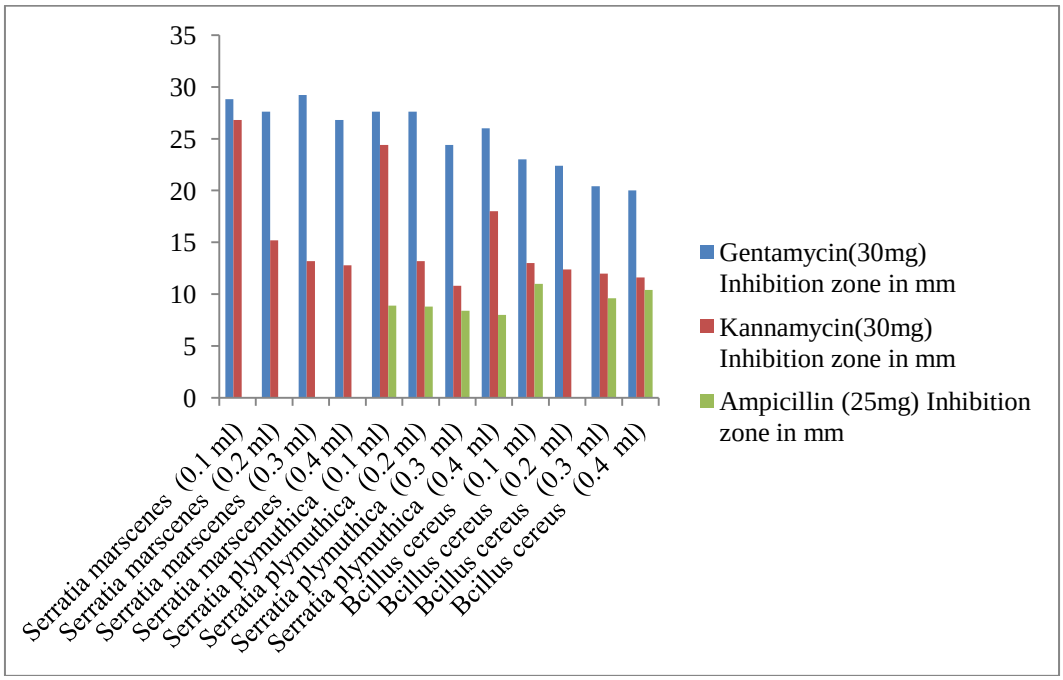
Serratia being a microaerophilic and rapidly growing organism producing different kinds of antibiotic degrading enzymes and secondary bactericidal agents like prodigiosin and different enzymes, which resist the host bactericidal activity and colonization of other bacteria in the host. These pathogenic bacteria activity will reduce food degradation competence in silkworm leading to uneconomical silk rearing. Even though silk worm produces bactericidal compounds to curtail pathogenic bacteria activity, lower concentration of compound and evolutionary adaptability of pathogenic bacteria circumvents bactericidal components of host. Determining

narrow spectrum antibiotics against septicemia pathogen perform bactericidal activity efficiently and reduce antibiotic resistant bacteria advancement. Bacteria colonizing *Bombyx mori* facilitate in digestion of the ingested food material by conglomerating with amylase, caseinase, gelatinase, lipase and urease (Ramesh, et al., 2009).

Serratia Sp., contains no plasmid and capsule to counter antibiotics effect but genome contains coding sequences for enzymes to degrade many of broad spectrum antibiotics. Due to microaerophilic and the rapid growing nature of Serratia, it hinders susceptibility tests during standardization, leading to variability in susceptibility. However, disk diffusion methods are suitable for detecting resistance to the commonly used antimicrobials against Serratia species. The conducted disk diffusion experiment is way out towards determining MIC for *Serratia* and *Bacillus* species.

In conducted experiment constant concentrations of frequent antibiotics were used with variable dilutions of pathogens. The inferences obtained from the disk diffusion experiment corresponds with the general hypothesis that, the number of bacteria increases efficiency of antibiotic decreases. Ampicllin being β lactam antibiotic shows meager activity on *Serratia plymuthica*, no activity for *Serratia marcescens* envisaging its futility towards G-ve bacteria. However, inhibition Bacillus cereus a gram positive bacteria indicate its prospective utilization against other G +ve bacteria. Also designates that the Serratia genome may code for pencillinase enzymes, which needs further insights. Gentamycin and Kanamycin are active bactericidal agents to G +ve and G –the bacteria; however rapid activity revealed by Gentamycin indicates preference in selection, but it may lead to futility Kanamycin and evolution of resistant bacteria against Kanamycin. The degree of infection and density of bacteria in septicemic disease of silkworm to be given due consideration while administering Gentamycin as a remedial agent. Kanamycin being moderate and valuable antibiotic adjacent to Gentamycin against G +ve and G –ve bacteria may surmount precincts of Gentamycin. Prudent prospects of experiment advantageous to mitigate the role of pathogens in causing septicemia of silkworm. Envisaging leeway shows the path towards cost effective, Eco friendly and safe way of handling opportunistic pathogens in human disease management.

Graph1: Effect of antibiotics on growth of Silkworm septicemia causing bacteria.



The graph for tested bacteria against antibiotics depicts linear decrease in inhibition zone with increase in bacterial density.

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ACKNOWLEDGEMENTS

This study was supported by Council of Scientific and Industrial Research, India through to carry out my research work.